# The molecular architecture of the plant nuclear pore complex.

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**REVIEW PAPER**

# The molecular architecture of the plant nuclear pore complex

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Running title: Plant NPC structure

Abbreviations: FG, phenylalanine-glycine; Nup, nucleoporin; NPC, nuclear pore complex

## Abstract

The nucleus contains the cell's genetic material, which directs cellular activity via gene regulation. The physical barrier of the nuclear envelope needs to be permeable to a variety of macromolecules and signals. The most prominent gateways for the transport of macromolecules are the nuclear pore complexes (NPCs). The NPC is the largest multiprotein complex in the cell, and is composed of multiple copies of approximately 30 different proteins called nucleoporins. Although much progress has been made in dissecting the NPC structure in vertebrates and yeast, the molecular architecture and physiological function of nucleoporins in plants remains poorly understood. In this review, we summarize the current knowledge regarding the plant NPC proteome and address structural and functional aspects of plant nucleoporins, which support the fundamental cellular machinery.

## Ultrastructure of the plant NPC

Because the nuclear pore complexes (NPCs) are the largest macromolecular complexes in cells, early studies of the complexes in plants were performed using electron microscopy. More than 40 years ago, Yoo and Bayley (1967) reported that nuclear pores in the pea plant resembled those described in various animal cells, and comprised 1 to 3 central granules (or possibly tubules), which were surrounded by an annulus. It was estimated that nuclear pores occupied a maximum of 38% of the nuclear envelope area. Roberts and Northcote (1970) used a freeze-etch technique to reveal the high-resolution structure of NPCs in sycamore and bean. They showed that, as in other organisms, the plant NPC is octagonally symmetrical around its cylindrical axis. They also determined that the plant NPC (1150 × 640 Å) is larger than its yeast counterpart (960 × 350 Å), but smaller than that in vertebrates (1450 × 800 Å). These studies provided the first model of higher plant NPCs, which was based on observation of vertical sections and a rough three-dimensional structure.

Fiserova *et al.* (2009) used an in-lens field emission scanning electron microscope (feSEM), a type of high-resolution SEM, to gain further insight into NPC structure. Using cultured tobacco cells, the NPC structure of the nuclear membrane was visualized on both the cytoplasmic and nucleoplasmic sides. They clearly demonstrated that, in both logarithmic and stationary phase cells, NPCs are non-randomly distributed over the nuclear envelope (as previously observed in other higher eukaryotes). The density of the NPCs was largely unchanged during cell growth, but NPCs became predominantly organized into rows in stationary phase cells. Interestingly, different NPC conformations, which were related to different cell stages, were observed. Logarithmic phase cells, which are metabolically active and undergo rapid cell division, contained NPCs with a larger inner pore diameter, which may be capable of rapid and effective transport. By contrast, stationary phase and senescent cells contained NPCs with smaller inner pore diameters; internal filaments

were observed within the pores, which emerged from the base of each subunit and were directed toward the NPC center. These results suggest that the NPC may differentially regulate transport activity and specificity by changing its component parts. Importantly, such a conformational change was also observed in *Xenopus* (Goldberg *et al.*, 1997) and *Drosophila* (Kiseleva *et al.*, 2001), suggesting that the mechanisms underlying NPC differentiation are conserved across eukaryotes.

Direct involvement of the inner nuclear envelope protein in NPC anchoring and positioning was suggested by studies in vertebrate cells (Lenz-Bohme *et al.*, 1997; Liu *et al.*, 2000; Maeshima *et al.*, 2006). The distribution of NPCs on the nuclear envelope in vertebrates correlates with the distribution of lamins. NPCs in tobacco are closely linked to a filamentous structure on the inner nuclear membrane (Fiserova *et al.*, 2009; Fiserova and Goldberg, 2010), and the organization and dimensions of these filaments resemble the arrangement of the nuclear lamina in *Xenopus* oocytes (Goldberg *et al.*, 2008). Although no lamin homologues have been identified in plants, the plant NPC might be anchored on the nuclear envelope in the same way as in vertebrates. Nuclear matrix constituent protein1 (NMCP1), which is a long coiled-coil protein localized at the nuclear rim, is considered to be the best candidate of plant lamin-like protein (Masuda *et al*., 1997; Boruc *et al*., 2012). Mutants of two NMCP1 homologues in *Arabidopsis, little nuclei1 (linc1)* and *linc2*, show reduced nuclear size and an altered nuclear structure (Dittmer *et al*., 2007). Thus, NMCP1/LINC is thought to determine nuclear organization in plants (Dittmer *et al*., 2007). Moreover, *Arabidopsis* SUN (Sad-1/UNC-84)-domain proteins, which are inner nuclear envelope proteins, have been isolated and characterized (Graumann et al., 2010; Oda and Fukuda, 2011). A mammal SUN-domain protein is known to interact with the NPC and likely regulates NPC distribution across the nuclear surface (Liu *et al*., 2007). It will be necessary to determine how plant NPCs interact with these proteins on the inner nuclear envelope if we are to better understand NPC function, positioning, assembly, and disassembly.

## Attempts to identify NPC components using proteomics

### *Yeast and vertebrate NPC proteomes*

The first comprehensive proteomics study of NPCs was performed in yeast (*Saccharomyces cerevisiae*) (Rout *et al.*, 2000). There are several advantages to working with yeast nuclei: yeast have the highest NPC/nuclear volume ratio of any organism (Maul, 1977) and, unlike the nuclei in mammalian cells, they do not have a lamina connecting the NPCs to other structures and/or protein complexes. Rout and Blobel (1993) prepared a highly enriched NPC fraction from yeast spheroplasts after several rounds of sucrose density gradient centrifugation. The fraction was then subjected to three different HPLC separation techniques followed by SDS-PAGE to identify the individual proteins associated with the NPC (Rout *et al.*, 2000). Matrix-associated laser desorption ionization time-of-flight (MALDI-TOF) and MALDI-ion trap tandem mass spectrometry identified a total of 174 proteins, of which 34 were previously uncharacterized ORFs. The uncharacterized ORFs and putative nucleoporins were epitope-tagged and their subcellular localizations were analyzed by immunofluorescence and immunoelectron microscopy to determine the position and stoichiometry of each nucleoporin within the NPC. In all, 29 nucleoporins and 11 NPC-associated proteins, which serve as transport factors, were identified.

Two years after this yeast NPC proteomics study, Cronshaw *et al.* (2002) reported the first vertebrate NPC proteomics data. They developed a fractionation procedure that yielded highly enriched nuclear envelopes from rat liver nuclei. After removing the chromatin, the nuclear membranes and their associated proteins were extracted by incubation in Triton X-100 and SDS. This procedure yielded intact NPCs embedded in the lamina. Then, a zwitterionic detergent was used to specifically solubilize the NPCs and release the monomeric nucleoporins. The solubilized proteins were separated by HPLC and SDS-PAGE and analyzed by using both

MALDI-quadrupole-quadrupole time-of-flight (MALDI-QqTOF) and MALDI-ion trap spectrometry. The uncharacterized proteins were then expressed as GFP fusions in HeLa cells to investigate their localization. This work identified and classified 29 nucleoporins and 18 NPC-associated proteins (Cronshaw *et al.*, 2002).

*The plant NPC proteome*

Knowledge of the individual components and overall structure of NPCs in plants largely lagged behind that of vertebrate and yeast NPCs. With a few exceptions, nucleoporin homologues could not be identified in plants using homology-based approaches (Meier, 2006). Several nuclear proteomics studies were reported, but they only identified a few nucleoporins (Pendle *et al.*, 2005; Aki and Yanagisawa, 2009). It was largely unknown which nucleoporins comprised plant NPCs. Because a protocol for the biochemical isolation of plant NPCs has not been developed, it is difficult to perform studies similar to those done in yeast and vertebrates. To overcome these problems, interactive proteomics was used to identify plant NPC components (Tamura *et al.*, 2010). Transgenic plants were generated, which expressed GFP-tagged mRNA export factor1 (RAE1), a known nucleoporin in *Arabidopsis*. The nucleoporins were then purified by immunoprecipitating the extracts from transgenic plants with an anti-GFP antibody. A linear ion trap mass spectrometer (LTQ-Orbitrap) was used to identify a total of 200 proteins in the immunoprecipitates. By comparing these with a database containing metazoan nucleoporins and performing expression studies of GFP fusions, 24 proteins were classified as nucleoporins (Tamura *et al*., 2010).

To obtain more information about plant NPCs, Tamura *et al.* (2010) selected other nucleoporins identified in the RAE1-GFP immunoprecipitates and used them as bait for further rounds of NPC purification. Finally, five cycles of interactive proteomic analysis were performed. This procedure identified at least 30 putative

nucleoporins, 22 of which had not been previously annotated. This work also demonstrated that the interactive proteomic approach is a very powerful technique, which can be used to comprehensively identify the individual components of macromolecular complexes in plants.

## Domain architecture

Proteomic analysis and x-ray crystallography revealed the detailed protein structure of individual nucleoporins. It was estimated that 38% of all nucleoporin amino acid residues contain an α-solenoid fold, 29% contain Phe-Gly repeats (FG repeats), and 16% contain β-propeller folds. Other individual fold types accounted for less than 5% of the total nucleoporin pool (Devos *et al.*, 2006). The small number of predicted fold types within the nucleoporin proteins and their similar internal symmetries suggest that the bulk of the NPC structures evolved through a series of gene duplications and divergences from a simple precursor set of only a few proteins. The predicted structure of individual *Arabidopsis* nucleoporins are summarised in Fig. 1.

### *Phe-Gly repeats*

There are an estimated 128 FG domains, which harbour thousands of total FG repeats, within any given yeast NPC (Rout *et al.*, 2000). The FG repeats interact with nuclear transport receptors, providing a selective barrier to the diffusion of macromolecules (Radu *et al.*, 1995; Patel *et al.*, 2007). The FG repeats appear to be localized toward the inside of the NPC (Rout *et al.*, 2000). It is therefore reasonable to suggest that nucleoporins rich in FG repeats (FG nucleoporins) coat the central pore surface, providing interaction domains for transport receptors within the central pore. Several studies investigated how these FG repeats function as a transport barrier. It appears that the FG repeats are intrinsically unfolded, and contain short

clusters of hydrophobic amino acids (e.g., FXFG or GLFG) separated by hydrophilic spacers. Because of flexibility of FG-repeat domains, it was proposed that phenylalanine-mediated inter-repeat interactions cross-linked the FG repeat domains into elastic and reversible hydrogels (Frey *et al.*, 2006). The hydrogel-like properties of the nuclear pores were thought to function as a molecular sieve. Systematic and large-scale analyses of the FG nucleoporins in yeast revealed that two forms are present in NPCs (Patel *et al.*, 2007). FG nucleoporins anchored at the NPC centre (central FG nucleoporins) form a cohesive meshwork of filaments via hydrophobic interactions. By contrast, FG nucleoporins anchored at the NPC periphery (cytoplasmic and nucleoplasmic FG nucleoporins) are generally noncohesive. Therefore, a two-gate model of NPC architecture is proposed, which comprises a central diffusion gate formed by a meshwork of cohesive FG nucleoporins and a peripheral gate formed by repulsive FG nucleoporins (Patel *et al.*, 2007; Strambio-De-Castillia *et al.*, 2010).

*Coiled coils*

The nuclear basket within the NPC is generally constructed from large coiled-coil proteins: NUCLEAR PORE ANCHOR (NUA) in plants (Xu *et al.*, 2007a), MYOSIN-LIKE PROTEIN1/2 (Mlp1/2) in yeast (Rout *et al.*, 2000), and TRANSLOCATED PROMOTER REGION (Tpr) in vertebrates (Cordes *et al.*, 1997). Coiled coils mediate protein-protein interactions, implying that the nuclear basket serves as a recruitment platform that brings various factors together within the nucleus. The yeast FG-nucleoporin complex, Nsp1-Nup82-Nup49 complex (Nup62-Nup58-Nup54 complex in plants), is held together by coiled-coil interactions (Bailer *et al.*, 2001) and tethered to the NPC scaffold via the N-terminal coiled-coil region of NUCLEOPORIN-INTERACTING COMPONENT OF 96 kDa (Nic96). The FG nucleoporins in plants also possess a coiled-coil domain and are thus assumed

also to form a complex (Nup62-Nup58-Nup54 complex). However, Nup93, a plant homologue of yeast Nic96, has no obvious coiled-coil motif (Tamura *et al*., 2010), implying that the plant FG-nucleoporin complex is tethered to the NPC scaffold via other interactions.

### α-*solenoids*

The α-solenoid fold comprises a two- or three- α helix unit, which is repeatedly stacked to form an elongated domain with the N- and C-termini at opposite ends of the molecule (Brohawn *et al.*, 2009). Approximately one-third of nucleoporins contain α-solenoid domains (Devos *et al.*, 2006). The outer and inner rings (scaffold nucleoporins) are dominated by an evenly distributed meshwork of α-solenoid domains, which is expected to facilitate the formation of a flexible fold. This allows large conformational changes without breaking protein-protein interactions, accommodates nucleocytoplasmic transport cargoes of different sizes, and promotes malleability of the nuclear envelope (Alber *et al.*, 2007a). α-solenoid folds are also common in large protein assemblies, such as those found in clathrin-, COPI-, and COPII-coated vesicles (Devos *et al.*, 2004). In coated vesicles, clathrin-like and adaptor proteins form the structural scaffold of the protein coat, which surrounds the membrane of the vesicle in a lattice-like fashion (Fotin *et al.*, 2004a; Fotin *et al.*, 2004b); a role that maybe similar to that played by the scaffold nucleoporins in the NPC. Therefore, it was proposed that the core structures of NPCs and vesicle-coating complexes have a common evolutionary origin (Devos *et al.*, 2004; Devos *et al.*, 2006).

### *β-propellers*

The β-propeller is a disc-like structure assembled from structural modules, known as

blades, circularly-arranged around a central channel (Chen *et al.*, 2011). In general, each blade consists of 4–10 repeats of a four-stranded antiparallel β-sheet motif. The central channel of the propeller fold is usually funnel-like in shape, with a wider bottom opening, which serves as entry point to the active site. A set of nucleoporins was initially identified as β-propellers based on sequence analysis. In yeast, only SECRETORY13 (SEC13) and SEC13 HOMOLOGUE1 (SEH1) contain the signature WD-40 repeat motif, and were among the very first β-propellers to be identified (Pryer *et al.*, 1993). Since then, other nucleoporins containing WD-40 repeats have been identified as β-propeller nucleoporins. Berke *et al.* (2004) experimentally determined the crystal structure of the N-terminal domain of human Nup133. They revealed that it consists of a seven-bladed β-propeller, which provides a molecular platform that mediates multiple interactions with other proteins (Berke *et al.*, 2004). After this structure was solved, additional noncanonical β-propeller domains within the NPC were identified. The β-propellers are widely assumed to serve as protein-protein interaction sites (Chen *et al.*, 2011). Peripheral β-propeller domains function to recruit accessory proteins, whereas those located centrally within the NPC bind subcomplexes (Strambio-De-Castillia *et al.*, 2010; Grossman et al., 2012).

The α-solenoid and β-propeller nucleoporins are thought to act as the structural scaffold of the NPC. Both the α-solenoid and the β-propeller folds provide extensive solvent-accessible surfaces, which appear well-suited for binding other proteins. Moreover, the α-solenoid and β-propeller folds seem to be quite robust and can tolerate significant variation in amino acid sequence while still retaining the core structure, thereby allowing optimization interactions with many different partners (Devos *et al.*, 2006).

## NPC architecture

Electron microscopy studies have dissected the NPC structure (Callan and Tomlin,

1950;Yoo and Bayley, 1967; Roberts and Northcote, 1970). These studies show that the general morphology of the NPC is conserved among eukaryotes. Using a different approach, *Alber et al.* (2007) performed a computational analysis to determine a detailed architectural map of the yeast NPC. They combined a diverse set of immunoelectron microscopic, crystallographic, and proteomic data to generate the map with an estimated 5 nm resolution, which agreed with a large body of complementary data for both vertebrates and yeast (Alber *et al.*, 2007a; Alber *et al.*, 2007b; Brohawn *et al.*, 2009). Although the primary sequence homology between nucleoporins from different model organisms is low, the high conservation of the overall shape and predicted fold types suggests that this NPC map can be applied to the NPCs of other organisms. Therefore, we compared a dataset derived from each plant nucleoporin with those from yeast and vertebrate nucleoporins and generated an architectural map of plant NPCs (Fig. 2). According to this model, nucleoporins can be subdivided into five classes: transmembrane ring, core scaffold (inner ring, outer ring, and linker), cytoplasmic filaments, nuclear basket, and central FG (Alber *et al.*, 2007a; Alber *et al.*, 2007b; Brohawn *et al.*, 2009; Grossman *et al*., 2012).

*Transmembrane ring nucleoporins*

Transmembrane nucleoporins are thought to anchor the NPC to the pore membrane and bind the assembled complex to the nuclear envelope. In plants, two transmembrane nucleoporins, GLYCOPROTEIN OF 210 kDa (Gp210) (Pom152 in yeast) (Gerace *et al*., 1982; Greber *et al*., 1990) and NUCLEAR DIVISION CYCLE1 (NDC1) (Wozniak *et al*., 1994), constitute an outer transmembrane ring. In addition to these proteins, yeast and vertebrates possess their own unique membrane proteins, PORE MEMBRANE PROTEIN OF 34 kDa (Pom34) (Rout *et al*., 2000) and Pom121 (Hallbereg *et al*., 1993), respectively. In vertebrates, Gp210 serves a fundamental role in NPC disassembly and is phosphorylated during nuclear envelope breakdown

(Galy *et al.*, 2008). An *Arabidopsis gp210* knockout mutant was identified as an embryo defective mutant (EMB3012) (http://www.seedgenes.org/index.html) (Meinke *et al.*, 2008), which arrested at the proglobular stage during embryogenesis. This suggests that plant Gp210 plays an essential role in embryonic development. Human NDC1 is involved in NPC assembly and is required for targeting the heritable disease-associated nucleoporin, Aladin, to the NPC (Kind *et al.*, 2009; Yamazumi *et al.*, 2009). It would be interesting to determine whether a plant Aladin also tethers to the NPC via NDC1.

*Cytoplasmic filaments and the nuclear basket*

Two characteristic peripheral NPC components, the cytoplasmic filaments and the nuclear basket, are localized asymmetrically within the cytoplasm and nucleoplasm of the NPC, respectively. These structures play a role in specific interactions that can serve as docking sites for transport complexes at both cytoplasmic and nucleoplasmic sides. The cytoplasmic filaments are composed primarily of two FG nucleoporins, Nup214 and CG1 (Kraemer *et al.*, 1995; Rout *et al.*, 2000). Although these nucleoporins are not well-characterized in plants, those in yeast provide sites at which mRNA export factors can maturate messenger ribonucleoprotein particles (Folkmann *et al.*, 2011). The biggest nucleoporin in vertebrates (Nup358) is localized in the cytoplasmic filaments, and tethers RanGAP (RanGTPase-activating protein) to the NPC to facilitate transportin-dependent nuclear import (Hutten *et al.*, 2009). By contrast, plants and yeast have no Nup358 homologues in their genomes (Grossman *et al.*, 2012). Plants use the WIP-WIT complex (discussed below) to anchor RanGAP on the nuclear envelope (Xu *et al.*, 2007b; Zhao *et al.*, 2008), whereas yeast RanGAP is localized to the cytosol (Hopper *et al.*, 1990).

The nuclear basket is composed of eight elongated filaments, which protrude ~60–80 nm from the nuclear face of the NPC into the nucleoplasm and

converge on a distal ring structure (Strambio-De-Castillia *et al.*, 2010). Each nuclear basket consists of one nucleoporin (NUA in plants), which has long coiled coil domains, and two FG nucleoporins (Nup136/Nup1 and Nup50 in plants). Human Tpr (NUA in plants) is thought to constitute the central architectural element that forms the scaffold of the nuclear basket, whereas Nup153 (Nup136/Nup1 in plants) binds to the nuclear coaxial ring linking the NPC core structures to Tpr (Krull *et al.*, 2004). It is reported that both *Arabidopsis* NUA and Nup136 are involved in mRNA export (Xu *et al.*, 2007a; Tamura *et al.*, 2010), as suggested by a study of vertebrate Tpr (Shibata *et al*., 2002). Importantly, *Arabidopsis nua* and *nup136* mutant plants display similar phenotypes, including early flowering and low fertility, indicating that NUA and Nup136 function together during plant development. In *Xenopus*, Nup153 (Nup136/Nup1 in plants) is the only nucleoporin that interacts with lamin B, which supports the nuclear structure. Although no lamin homologue has been identified in plants, it is reported that *Arabidopsis* nuclei can change shape, depending on the amount of accumulated Nup136 (Tamura *et al.*, 2010; Tamura and Hara-Nishimura, 2011). This implies that Nup136/Nup1 is the nucleoporin that links the NPC to the nuclear lamina in plants.

*Central FG nucleoporins*

Central FG nucleoporins, also known as barrier nucleoporins, show a transverse orientation with respect to the central channel of the NPC and form a selective barrier that mediates nucleocytoplasmic transport. Native FG repeat regions have an unfolded structure, allowing multiple low-affinity and high-specificity interactions with transport factors (Strawn *et al.*, 2004). In fact, *in vitro* studies show that all FG nucleoporins appear to have the ability to interact with at least one transport receptor (Ryan and Wente, 2000). Bayliss *et al.* (2000) determined the crystal structure of the human transport receptor, importin β, and identified its two FG repeat binding sites. It

is estimated that there are approximately 160 transport factor binding sites within each NPC (Grossman *et al.*, 2012), which allow the simultaneous binding of multiple transport factors within a single NPC.

Five central FG nucleoporins (Nup98, Nup62, Nup58, Nup54, and Nup35) have been identified in plants and are well conserved in other organisms. Of these, only Nup62 has been characterized in *Arabidopsis* (Zhao and Meier, 2011). *Arabidopsis* Nup62 interacts with nuclear transport factor2 (NTF2), as previously observed in yeast (Nsp1) (Clarkson *et al*., 1997) and vertebrates (Nup62) (Percipalle *et al.*, 1997). Knockdown of Nup62 transcripts results in a severe dwarf phenotype and early flowering, indicating an important function of Nup62 at different stages of plant development. Further analysis of central FG nucleoporins will be required to identify how the different FG nucleoporins function during the plant life cycle. This will increase our understanding of the specific interactions between transport factors and NPCs in plants.

*Outer ring, inner ring, and linker nucleoporins (scaffold nucleoporins)*

Scaffold nucleoporins are composed of three groups of nucleoporin subcomplexes: the outer ring, the inner ring, and the linker. They connect membrane nucleoporins to central FG nucleoporins thereby bridging the anchoring transmembrane layer and the barrier layer of the NPC. Because scaffold nucleoporins form the rigid skeleton of the NPC, most remain incorporated within the NPC during the entire life of a cell (D'Angelo *et al.*, 2009). Scaffold nucleoporins are thought to play a key role in maintaining the stability of the nuclear envelope by ensuring co-planarity of the outer and inner surfaces (Alber *et al.*, 2007a). Therefore, it is proposed that a major function of the outer rings is to facilitate the smooth transition of the pore membrane into the inner and outer nuclear envelopes (Alber *et al.*, 2007a).

The largest and most evolutionarily conserved subcomplex, comprising

several nucleoporins, is located at the outer rings and is known as the Nup107/160 subcomplex in plants and vertebrates and the Nup84 subcomplex in yeast. In plants, it is composed of eight nucleoporins: Nup160, Nup133, Nup107, Nup96, Nup85, Nup43, SEC13, and SEH1 (Fig. 2) (Xu and Meier 2007; Tamura *et al*., 2010; Wiermer *et al*., 2012). Electron microscopic and biochemical analyses revealed that the yeast Nup84 subcomplex has a 40-nm long, Y-shaped, triskelion-like structure (Siniossoglou *et al.*, 2000; Kampmann and Blobel, 2009). This structure has two hinge regions that are conformationally flexible, which allows the NPC scaffold component to change its structure to enable large cargoes to pass through the central channel (Kampmann and Blobel, 2009). The scaffold nucleoporins located in the central part of the main channel are called inner ring nucleoporins and linker nucleoporins. In plants, the inner ring consists of Nup205, Nup188, Nup155, and Nup35 (Fig. 2) (Tamura *et al*., 2010). Similar to the outer ring nucleoporins, they contain α-solenoid and β-propeller folds and exhibit the typical structural scaffolding motif. The linker nucleoporins are attached between the outer and inner rings (Alber *et al.*, 2007a), and include Nup93 and Nup88. They act as a bridge between the core scaffold and the FG nucleoporins.

Genetic studies have characterized several plant scaffold nucleoporin mutants. *Arabidopsis nup96* and *nup88* mutants were isolated during a genetic screen aimed at identifying downstream components responsible for resistance (R) protein activation (Zhang and Li, 2005; Cheng *et al.*, 2009). Both mutants showed deficiencies in innate immunity and, possibly, an impairment in nuclear import of proteins, which is involved in the plant response to pathogens. Furthermore, Wiermer *et al.* (2012) isolated knockout mutants for each component of the Nup107/160 complex and investigated plant immune responses. Of the seven mutated nucleoporins, only *nup160* and *seh1* caused impaired immune responses. These results suggest that Nup160, Nup96, and SEH1 within the Nup107/160 subcomplex are important for defense signaling (Wiermer *et al.*, 2012). The other Nup107/160

subcomplex proteins, Nup133 and Nup75, are required for fungal and rhizobial colonization in *Lotus japonicus* (Kanamori *et al.*, 2006; Saito *et al.*, 2007). Moreover, *Arabidopsis nup160* and *nup96* mutants show altered hormonal and temperature responses and an early flowering phenotype (Dong *et al.*, 2006; Parry *et al.*, 2006). Further work is required to identify the underlying molecular mechanisms and functions of the plant Nup107/160 subcomplex in various signaling pathways.

**NPC-associated proteins**

*TREX-2*

The TREX-2 (transcription-coupled export 2) complex comprises SUPPRESSOR OF ACTIN3 (SAC3), Tho2/Hpr1 PHENOTYPE1 (THP1), SL GENE UPSTREAM OF ySa1 (SUS1), and CELL DIVISION CYCLE31 (CDC31) proteins, and is involved in mRNA export in yeast (Kohler and Hurt, 2007). The TREX-2 complex anchors at the inner side of the NPC via Nup1 (Nup136/Nup1 in plants) and Nup60 (Nup50 in plants) (Fischer *et al.*, 2002). SUS1 also interacts with the SAGA (Spt, Ada, Gcn5 and acetyltransferase histone acetyltransferase) complex, a large transcription initiation complex that catalyzes histone acetylation and de-ubiquitylation. The TREX-2 complex is thought to functionally couple SAGA-dependent gene expression to mRNA export on the inner side of the NPC (Rodriguez-Navarro *et al.*, 2004). Lu *et al.* (2010) identified TREX2 components in *Arabidopsis*, including THP1, SUS1, SAC3s, and CDC31s, and showed that the Nup136/Nup1-THP1 interaction links the TREX-2 complex to the nuclear basket (Fig. 3). Using yeast two-hybrid assays and a bimolecular fluorescence complementation assay, they also showed that THP1 interacts with DSS1, a subunit of the 26S proteasome regulatory particle (Lu *et al.*, 2010). Consistent with this finding, yeast SDD1 was identified as a functional component of the TREX-2 complex (Mannen *et al.*, 2008), and is required for nuclear export of specific sets of mRNA. These results suggest that plants utilize

413 an evolutionarily conserved system for mRNA export through the NPC.

414

415 *ESD4*

416 In *Arabidopsis,* EARLY IN SHORT DAYS4 (ESD4) functionally interacts with NUA, a
417 major component of the nuclear basket (Xu *et al.*, 2007a). Genetic analysis indicates
418 that NUA and ESD4 might act via a shared pathway involved in various aspects of
419 plant development. ESD4 encodes a SUMO (small ubiquitin-related modifier
420 conjugates)-specific protease and is localized on the nuclear envelope (Murtas *et al.*,
421 2003) (Fig. 3). In both *esd4* and *nua* mutants, the accumulation of SUMO conjugates
422 increases, suggesting that ESD4 and NUA are involved in SUMO homeostasis and in
423 regulating nucleocytoplasmic transport in plants. The nuclear basket components,
424 Mlp1/2 in yeast and Nup153 in humans, tether the SUMO-conjugating enzymes,
425 UBIQUITIN-LIKE PROTEIN1 (Ulp1) and SENTRIN-SPECIFIC PROTEASE2
426 (SENP2), respectively (Zhang *et al.*, 2002; Panse *et al.*, 2003). The yeast *ulp1*
427 mutant displays altered protein sumoylation patterns and increased pre-mRNA
428 leakage into the cytoplasm. These results suggest that desumoylation within the
429 NPC might be a key regulatory event that prevents inappropriate pre-mRNA export in
430 different species (Lewis *et al.*, 2007).

431

432 *WPP DOMAIN-INTERACTING PROTEIN (WIP) and WPP DOMAIN-INTERACTING*
433 *TAIL-ANCHORED PROTEIN (WIT) complexes*

434 In vertebrates, RanGAP, which plays an important role in nucleocytoplasmic
435 transport, is anchored to the NPC via Nup358 (Strambio-De-Castillia *et al.*, 2010).
436 This interaction is mediated by a unique C-terminal domain within RanGAP, and is
437 dependent upon sumoylation. By contrast, plant NPCs lack a Nup358 homologue,
438 and all known plant RanGAPs contain a unique N-terminal domain called the WPP

domain (a highly conserved Trp-Pro-Pro motif), which is necessary and sufficient for nuclear envelope targeting (Meier, 2000). Two types of plant-specific nuclear envelope proteins, WIPs and WITs, were isolated and identified as RanGAP-anchoring proteins (Xu *et al.*, 2007b; Zhao *et al.*, 2008). Although there is no direct evidence that WIP and WIT proteins physically interact with the NPC, it is assumed that they have a functional connection (Fig. 3). Recently, *Arabidopsis* WIP was reported to function as a KASH (Klarsicht/ANC-1/Syne Homology)-domain protein, which interacts with a SUN-domain protein (Zhou *et al.*, 2012). This SUN-KASH bridge is necessary for maintaining the elongated nuclear shape of epidermal cells, indicating that WIPs are versatile and play different roles.

## Conclusion

Since the identification of the plant NPC proteome, much progress has been made in clarifying the molecular components and architecture of the NPC. Despite conserved functional similarities between NPCs from plants and other organisms, structural differences exist. It is necessary to determine how these structural variations and protein sequence differences contribute to the functional organization of the plant NPC. Clearly, reverse genetic studies and high-resolution imaging techniques are needed if we are to understand the function and structure of each nucleoporin in detail.

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## Figure legends

**Fig. 1.** Summary of *Arabidopsis* nucleoporins. The structural motifs that appear next to each nucleoporin refer to the yeast protein folds predicted by Devos *et al.* (2006). Protein fold illustrations were derived from PDB ID: 1D7M, 1DXZ, 1XKS 2Q5X, 2RFO, and 3T97. AGI code: *Arabidopsis* genome initiative code.

**Fig. 2.** Molecular architecture of the NPC based on the model proposed by Alber *et al.* (2007a). The nucleoporins (Nups) are grouped according to their location. The NPC is divided into seven component groups. The symmetrical core is composed of the outer ring Nups, the linker Nups, the inner ring Nups, the transmembrane ring Nups, and the central FG Nups. The asymmetric parts of the pore are formed by the cytoplasmic FG Nups and filaments and the nuclear FG Nups and basket.

**Fig. 3.** NPC-associated proteins in plants. The plant NPC associates with many proteins, including the TREX-2 complex, ESD4, and the RanGAP-WIT-WIP complex. This allows the NPC to participate in diverse cellular functions.


| Name | AGI code |
|---|---|
| Nup160 | At1g33410 |
| Nup155 | At1g14850 |
| Nup133 | At2g05120 |
| Nup107 | At3g14120 |
| Nup96 | At1g80680 |
| Nup75 | At4g32910 |
| Nup205 | At5g51200 |
| Nup188 | At4g38760 |
| Nup93 | At2g41620/At3g57350 |
| Nup43 | At4g30840 |
| SEH1 | At1g64350 |
| SEC13 | At2g30050 |
| Aladin | At3g56900 |
| RAE1 | At1g80670 |
| Nup88 | At5g05680 |
| NUA | At1g79280 |
| Nup136/Nup1 | At3g10650 |
| Nup98 | At1g10390/At1g59660 |
| Nup50 | At1g52380/At3g15970 |
| CG1 | At1g75340 |
| Nup62 | At2g45000 |
| Nup58 | At4g37130 |
| Nup54 | At1g24310 |
| Nup214 | AAD10642 |
| gp210 | At5g40480 |
| NDC1 | At1g73240 |
| Elys/HOS1 | At2g39810 |
| GLE1 | At1g13120 |
| Nup35 | At3g16310 |

Figure 1. Tamura and Hara-Nishimura

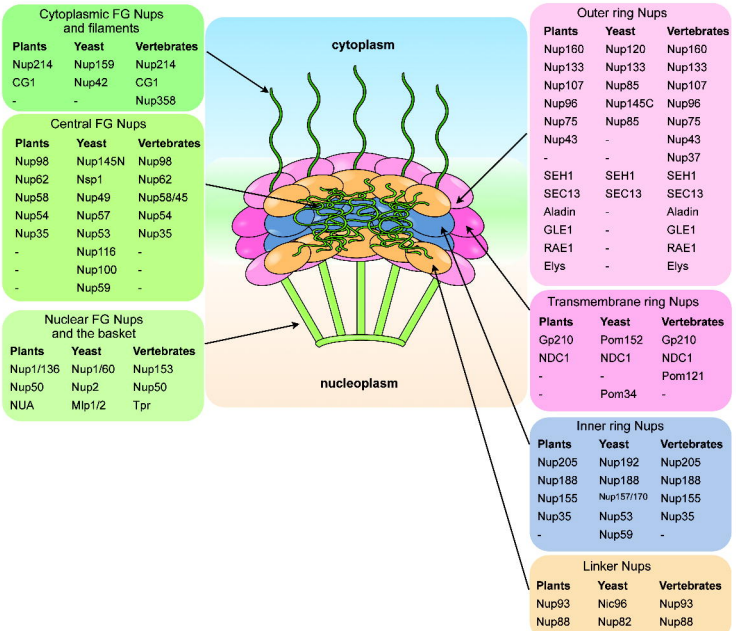


Figure 2. Tamura and Hara-Nishimura

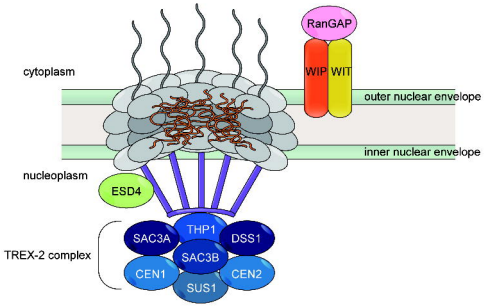


Figure 3. Tamura and Hara-Nishimura